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Effect of Alcalase Hydrolysis Time on Physicochemical and Functional Properties of Protein Hydrolysate Extracted from *Gryllus bimaculatus* Cricket Powder

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Abstract

Humans are increasingly consuming insects as an alternative source of protein, with various products being developed to improve their acceptability. Insect protein extracts are becoming notable food ingredients, with a few studies investigating the functional and biological properties of proteins from different insects. This study determined the effect of hydrolysis time on the functional, chemical, and biological activities of cricket protein hydrolysate (CPH) extracted from *Gryllus bimaculatus*. The cricket powder was hydrolyzed using the enzyme alcalase at 4% w/w. The hydrolysate samples were collected at 20, 40, 60, 120, and 180 min and analyzed for many properties. The CPH obtained at 120 and 180 min had the highest degree of hydrolysis (DH) (84.42-84.78%), while the CPH obtained from 60-180 min hydrolysis had the highest yield (34.92-36.99%). Free amino

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acids increased over time. The CPH powder was highly soluble at pH 7 and had higher foaming ability and stability than the control, while the emulsion abilities of the CPH and the control were similar. The CPH obtained after 120 to 180 min of hydrolysis showed the strongest antioxidant activity analyzed using the DPPH, FRAP, and ABTS assays. CPH after 120 min of hydrolysis showed the highest ACE inhibition ability of 67.59%. The results suggested that CPH prepared by hydrolyzing cricket powder with alcalase for 60 and 120 min was suitable and showed potential as a protein source with high water solubility and good foaming ability for further non-food applications.

Abbreviations: CPH = Cricket protein hydrolysate; DH = Degree of hydrolysis; DPPH = Diphenyl-2-picrylhydrazyl assay; ABTS = 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay; FRAP = Ferric reducing antioxidant power; ACE = Angiotensin-converting enzyme

Introduction

Edible insects are becoming increasingly appreciated as an alternative to high-protein food, with consumers preferring processed insect products.[1] Developing foods to persuade consumers to accept and eat more insects has become a major challenge, especially in regions where the consumption of whole insects lacks sensory appeal. When cricket powder is processed into hydrolyzed protein, it has a higher water solubility and can be used in a wider range of foods. Hydrolyzed protein is produced using different methods to hydrolyze proteins into short peptides and free amino acids. The structures, characteristics, and chemistry of proteins are all impacted by degradation. Acids and alkalis are commonly used as a quick and inexpensive method to hydrolyze the degradation of protein peptide bonds. However, chemicals used during production must be removed before use and may leave residue. Enzymatic analysis can improve the properties of the protein by using mild conditions and controlling the level of digestibility. The rate of decomposition using this method is also high compared to using acids and alkalis. Enzymatic hydrolysis of insect protein is an alternative method for improving the properties of its protein hydrolysate. Enzymatic hydrolysis can enhance protein extraction yield, increase the degree of hydrolysis (DH), and liberate free amino acids. Hydrolyzing *Tenebrio molitor* protein demonstrated improved yield, higher DH, and greater release of free amino acids.[2] Additionally, treatment with alcalase and flavoenzyme further enriched the amino acid profile and enhanced the product's scavenging potential.[3] Alcalase hydrolysis was also used to improve the technological and functional properties of protein flour produced from migrating locusts (*Locusta migratoria* L.).[4] Therefore, enzymatic hydrolysis is an alternative process for high protein edible insects, improving the functional and health-promoting properties of the resulting protein hydrolysates Crickets are a popular insect with protein quality and digestibility comparable to chicken, pork, and beef.[5] In particular field cricket (*Gryllus bimaculatus*) has a high protein concentration of approximately 60 to 70% dry weight, which is comparable to traditional animal proteins.[6] The nutritional value and practical uses of cricket components as food ingredients have recently attracted increased attention but few studies have addressed the functional characteristics of proteins from different insects. Insect hydrolysate effectiveness is contingent on protein characteristics such as high solubility and gel, foam, or emulsion formation stability. Protein hydrolysates can be applied across domains, including functional attributes like foams and emulsifiers. Using protein hydrolysates from edible crickets as an alternative protein source is a desirable choice in the context of global food security and nutrition. However, there is no information available about hydrolyzing edible cricket proteins with commercial enzymes such as alcalase, as well as the biological properties of protein hydrolysate produced. The purpose of this work was to investigate the effects of alcalase hydrolysis time on the physicochemical and functional properties of protein hydrolysates extracted from *G. bimaculatus* cricket powder. The results were compared to an enzyme-free protein hydrolysate.

Materials and Methods

Chemicals

Unless otherwise specified, all chemicals used were of reagent grade and sourced from Sigma Aldrich (St. Louis, MO, USA) and Thermo Fisher Scientific (Waltham, MA, USA). Alcalase® (a protease derived from *Bacillus licheniformis*, P2.4 U/g) was obtained from Sigma Aldrich. Angiotensin-converting enzyme (ACE) from rabbit lung and the substrate hippuryl-L-histidyl-L-leucine (HHL) were procured from Dinojo, Japan.

Raw Materials

Frozen *Gillus bimaculatus* were purchased from a local cricket farm in Maha Sarakham Province, Thailand. The crickets were cultivated under good agricultural practice (GAP) guidelines and harvested as adult crickets at 42-45 days old.[7]

Cricket Powder Preparation

After thawing and blanching the frozen crickets in a 1:10 (w/w) ratio for 10 min at 80°C, they were cooled in water at 10±1°C. A tray dryer was used immediately to dehydrate them. Three kilograms of crickets were divided equally among the drying trays and dried at 80°C (Memmert, OLM-500, Germany) until the water activity (a_w) was less than 0.6 and the moisture content was 10±1% (wet basis). Using the Kjeldahl method, the protein content of the finely ground powdered dried crickets was found to be 62.15%.[8]

Cricket Protein Hydrolysate Preparation

Cricket protein hydrolysate was produced with minor alterations following previous procudure.[9] The cricket powder (14.0 g) was dissolved in 70 mL of 0.2 M phosphate buffer (RCI Labscan, USA) at pH 8, and then added to alcalase enzyme (Sigma, USA) at a concentration of 4% by weight of protein in the powder (60.31%). Hydrolysis was carried out in a continuously shaking water bath (Lauda, Germany) at 50°C for 180 min, with samples collected at 0, 20, 40, 60, 120, and 180 min before the digestion was ended by heating to 95°C for 10 min. The samples were centrifuged for 30 min at 4,000 rpm using a Hettich centrifuge (UNIVERSAL 320). The clear solution (supernatant) was frozen at -30°C and then freeze-dried to obtain cricket protein hydrolysate (CPH). All experiments were conducted in triplicate.

Determination of Degree of Hydrolysis

The DH was measured using the hydrolysis rate of o-phthaldialdehyde (OPA).10 The protein hydrolysate powder was dissolved in distilled water (1 mg/mL) and 25 µL of protein hydrolysate solution was added to 188 µL of OPA reagent (Sigma, USA) and mixed at 25°C for 2 min. The absorbance of the mixture solution was recorded using a microplate reader (Spectrostar Nano, Germany) at 340 nm. The rate of degradation was computed using the equation:

$$\text{Degree of hydrolysis (DH)} = \frac{L_t - L_o}{L_{max} - L_o} \times 100$$

where DH (%) represents the degradation rate percentage,

L_t = the concentration of amino acids presents at different digestion times of protein hydrolysate.

L_o = the concentration of amino acids presents at the start of the mixture of cricket powder and phosphate buffer.

L_{max} was determined by measuring the total quantity of amino acids after the cricket powder was completely hydrolyzed with 12 N HCl for 24 hours (1:1) at 90°C.

Amino Acid Content Analysis

Each 25 mL tube was filled with a 1.0 mL aliquot of CPH solution (1% in water), followed by the addition of 0.8 mL of deionized water, 1.0 mL of phosphoric acid buffer solution (pH 6.0), and 1.0 mL of 2% ninhydrin solution. After 15 min of reaction at 90°C, the mixtures' volume was adjusted to 25 mL using deionized water. After measuring the solutions' absorbance at 570 nm, the free amino acid (FAA) content was computed using leucine-prepared standard curves.

Proximate Composition of Cricket Powder

The proximate compositions of CPH were determined using the AOAC official method (2000).[8]

Protein Content of Liquid Cricket Protein Hydrolysate

The protein contents of cricket hydrolysate samples collected at different hydrolysis times were determined by the Lowry method.[11]

Protein Solubility (%)

The CPH solubility was assessed using the modified method.[9] 200 mg of CPH and the control samples were dissolved in 20 mL of pH 7.0 buffer and agitated with a magnetic stirrer for 30 min at room temperature. After 15 min at 4°C, the mixture was centrifuged at 10,000 ×g. The protein content of the supernatant and the total protein content of each sample were determined using the Lowry method. Using the following formula, protein solubility was calculated and expressed as a percentage.

$$Solubility\ (\%) = \left(\frac{\text{Protein content in } supernatant}{Total\ protein\ content\ in\ sample}\right) x100$$

Foaming Ability and Foam Stability

The aeration method was utilized to assess foaming ability (FA). A 25 mL of phosphate buffer (pH 7.0) was used to dissolve a 4 mL aliquot of CPH. A stir bar was used to equilibrate the mixture for 10 min at room temperature. A homogenizer set to 12,000 rpm for 2 min was then used to aerate the mixture and produce bubbles (IKA, USA).4 The volume of the foam produced was measured by pouring it into a 25 mL measuring cylinder. The proportion of foam that remained after 30 min was used to compute the foaming ability and foaming stability (FS) as follows:

$$\text{Foaming ability (\%)} = \left(\frac{\text{Volume after whipping at 0 min(mL)} - \text{Volume before whipping (mL)}}{\text{Volume before whipping (mL)}}\right) \times 100$$

$$\text{Foaming stability (\%)} = \left(\frac{\text{Volume at 30 min(mL)} - \text{Volume before whipping (mL)}}{\text{Volume before whipping (mL)}}\right) \times 100$$

Emulsifying Activity

The emulsifying activity (EA) was evaluated using a previously reported method with minor modifications.[4] Briefly, Protein solutions (4%) were made by mixing CPH and the control sample with phosphate buffer pH 7.0. The samples were centrifuged at 3,388×g for 15 min after being allowed to equilibrate for two hours at room temperature. The supernatant was homogenized for one min at 12,000 rpm after being mixed with pure sunflower oil (1:1 v/v). Using scaled tubes, 15 mL aliquots of the emulsion were centrifuged at 3,388 ×g for 15 min at room temperature. The height of the overall height of the solution (HS) and the height of the resulting emulsified layer (HEL) were used to calculate the EA (Eq. 4) as follows:

EA (%) = (HEL/HS) × 100

Determination of Antioxidant Activity

The CPH samples were treated with and without enzyme hydrolysis at various interval periods.[7] A 0.3 g portion of each sample was extracted in 25 mL of 80% ethanol at 37°C for 18 hours using a shaker (Mettler Toledo, AG8603 Schwerzenbach). The mixture was filtered via filter paper after being centrifuged for 30 min at 4,000 ×g. The total phenolic content (TPC) and antioxidant activity of the extract samples were assessed.

Diphenyl-2-picrylhydrazyl (DPPH) Assay

DPPH radical scavenging activity was assessed.[12] The results were compared with a reference standard (gallic acid) and expressed as mg GAE/g dry basis of a sample.

2,2-azino-*bis*-3-ethylbenzothiazoline-6-sulphonic Acid (ABTS) Radical Scavenging Assay

The ABTS radical cation decolorization assay was used to measure the free radical scavenging activities of CPH and the control.[13] The results were reported in mg GAE/g dry basis of a sample.

Ferric Reducing Antioxidant Power (FRAP)

The ability of the CPH and control samples to change Fe^{3+} -TPTZ into blue-colored Fe^{2+}-TPTZ was used to calculate their reducing power.[14] The results were expressed in μM $FeSO_4$/g dry basis of material, and the absorbance was recorded at 539 nm.

Total Phenolic Content (TPC) Measurement

The TPC of the extracts was evaluated using the Folin-Ciocalteu reagent.[15] At 765 nm, the absorbance was determined with a spectrophotometer (CE 2041, England). Gallic acid was used to create the standard curve, and the results were reported as GAE/g dry basis of the sample.

Determination of Angiotensin-Converting Enzyme

An ACE Kit-WST (Dojindo Laboratories Technologies Inc., Japan) was used to evaluate the angiotensin-converting enzyme (ACE).[16] Enzyme B was

dissolved in 2 mL of distilled water to create the enzyme working solution, which was then added to enzyme A in 1.5 mL. Enzyme C and the coenzyme were dissolved in 3 mL of purified water and thoroughly mixed to create the indicator working solution. After adding 20 µL of each of the sample extract, substrate buffer, and enzyme working solution to a 96-well plate, the plates were incubated for one hour at 37°C (model IPS 260/750 Memmert: Germany) before 200 mL of the indicator working solution was added. A microplate reader was used to measure the sample mixture's absorbance at 450 nm after it had been left in the dark at room temperature for 10 min. The following formula was then used to determine the ACE inhibition ability:

$$\text{ACE inhibition (\%)} = \left[\frac{(A_{blank1}-A_{sample}-A_{sample\ blank})}{(A_{blank1}-A_{blank2})}\right]\times 100$$

Statistical Analysis

The results were presented as mean and standard deviation values for triplicate experiments. A one-way analysis of variance (ANOVA) was used to compare data from *G. bimaculatus* protein hydrolysis with and without alcalase at different times (0, 20, 40, 60, 120, and 180 minutes). Significant differences between treatments were identified using Duncan's multiple range test. The threshold for statistical significance was established at $p<0.05$. All experiments were completed in triplicate.

Results

Effect of Hydrolysis Time on Yields

Before hydrolysis, *G. bimaculatus* powder contained 62.15% protein and 20.22% fat. Table 1 displays the yields of CPH treated with and without alcalase hydrolysis (control) obtained from different hydrolysis times. The CPH yields increased significantly ($p<0.05$) when increasing the enzymatic hydrolysis duration, but no significant difference was recorded for yields in crickets without alcalase hydrolysis. Crickets hydrolyzed with alcalase for 120 and 180 min produced the highest yields of CPH (37.98% and 39.17%, respectively) while yields of crickets digested without adding enzyme varied from 23.35 to 25.05%.

Table 1: CPH yield, hydrolysis degree, protein, amino acids, and phenolic compounds with/without alcalase at different times

Treatment	Time (min)	Yield (%)	DH (%)	Protein (mg/mL)	Free amino acid (mg/g)
Control	0	23.85±0.04^{e}	13.62±0.02^{e}	10.14±0.67^{e}	1.63±0.04^{f}
	20	25.05±0.02^{e}	16.00±0.03^{d}	10.26±0.28^{e}	1.71±0.06^{e}
	40	23.35±0.04^{e}	18.69±0.02^{c}	10.96±0.67^{e}	1.91±0.01^{d}
	60	24.16±0.09^{e}	19.35±0.01^{b}	11.01±1.38^{e}	1.97±0.01^{d}
	120	23.62±0.01^{e}	21.39±0.01^{c}	10.74±0.25^{e}	2.07±0.02^{cd}
	180	24.43±0.04^{e}	27.46±0.02^{a}	10.85±0.40^{e}	2.11±0.07^{c}
Alcalase	0	24.15±0.04^{e}	48.91±1.17^{e}	12.38±0.30^{e}	1.72±0.06^{f}
	20	32.41±0.08^{d}	65.07±1.28^{d}	22.81±0.15^{d}	2.19±0.01^{c}
	40	33.97±0.14^{c}	75.50±0.82^{c}	25.06±0.46^{c}	3.32±0.03^{b}
	60	36.25±0.05^{b}	79.81±1.17^{b}	29.96±1.88^{b}	4.73±0.06^{a}
	120	37.98±0.21^{ab}	84.78±1.35^{a}	31.12±0.64^{a}	4.88±0.04^{a}
	180	39.17±0.19^{a}	84.42±1.21^{a}	31.67±1.44^{a}	4.94±0.06^{a}

Values are the means ± SD of dry weight samples taken in triplicate.
a-f significant differences are shown by different superscripts within a column ($p<0.05$).

Effect of Hydrolysis Time on DH

Alcalase hydrolysis time significantly affected the DH of *G. bimaculatus* crickets ($p<0.05$) (Table 1). Longer enzyme hydrolysis time yielded peptides with higher DH. Crickets hydrolyzed with alcalase for 120 and 180 min showed significantly ($p<0.05$) higher DH values at 84.78% and 84.42%, respectively whereas lower DH values were recorded in crickets without alcalase hydrolysis (control) ranging from 13.62 to 27.46%.

Effect of Hydrolysis Time on Protein and Free Amino Acid Content

Table 1 shows the change in protein and free amino acid (FAA) contents of CPH obtained from each hydrolysis time during 180 min13.62 of hydrolysis. The protein and FAA contents in CPH gradually increased at longer alcalase hydrolysis times, whereas changes in the control samples were not significantly different.

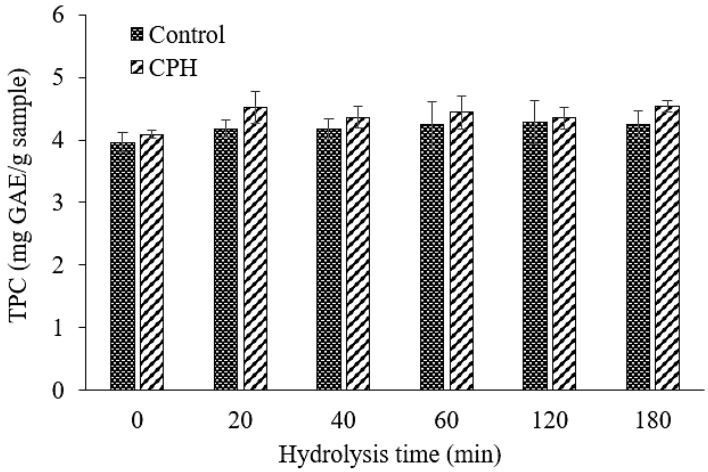

Fig. 1: Comparison of TPC at different hydrolysis times between hydrolyzed cricket protein and control

Effect of Hydrolysis Time on TPC

No significant impact ($p<0.05$) on TPC was determined at different hydrolysis times. TPC in the control samples treated without alcalase was comparable to values in CPH produced by an enzymatic process (Figure 1).

Table 2: Protein solubility, foaming properties, and emulsion activity of CPH with/without alcalase at different times

Treatment	Time (min)	Protein solubility (%)	Foaming properties		Emulsion activity (%) ^{ns}
			FA (%)	FS (%)	
Control	0	21.25±1.2^{g}	44.0±1.4^{e}	40.4±1.5^{e}	51.6±0.9
	20	23.16±1.7^{f}	44.0±2.8^{c}	56.0±1.0^{cd}	52.4±2.1
	40	23.67±2.8^{f}	42.5±0.7^{d}	55.5±0.7^{cd}	51.6±2.1
	60	25.12±1.2^{f}	40.3±1.4^{f}	56.7±1.0^{e}	51.4±0.6
	120	32.55±1.7^{e}	42.6±1.1^{b}	60.2±1.4^{c}	51.1±1.3
	180	33.14±1.7^{e}	40.2±1.2^{d}	52.0±0.7^{d}	51.6±0.9
Alcalase	0	29.78±1.08^{e}	96.7±1.8	80.0±0.9^{b}	50.9±0.7
	20	45.97±0.9^{d}	98.4±2.6	82.1±3.2^{b}	49.5±2.2
	40	66.51±1.5^{c}	99.0±1.4	84.7±2.1^{ab}	51.4±0.7
	60	76.16±3.3^{b}	100.0±3.1	86.4±1.7^{a}	52.0±1.0
	120	86.73±1.1^{a}	99.1±2.0	86.9±2.9^{a}	51.2±1.4
	180	85.34±2.8^{a}	98.3±2.5	86.1±2.7^{a}	51.1±1.3

Values are means ±SD of triplicate samples (dry weight).

a-f Different superscripts within a column indicate significant differences ($p<0.05$).

FA = foaming ability; FS = foaming stability.

Effect of Hydrolysis Time on Protein Solubility

Protein solubility at pH 7 gradually increased at longer hydrolysis times (Table 2). The highest protein solubility at 86.73% was recorded after 120 min, significantly higher than the control at the same hydrolysis time.

Effect of Hydrolysis Time on Foaming Properties

Foaming ability varied from 96.7 to 100% (Table 2), with the highest value recorded for alcalase CPH digestion at 60 min. Foaming stability ranged from 80.0 to 86.9% and was significantly higher than the control.

Effect of Hydrolysis Time on Emulsification Activity

The emulsification activity of CPH is shown in Table 2. EA values of all treatments were not significantly different and ranged from 49.5 to 52.4%.

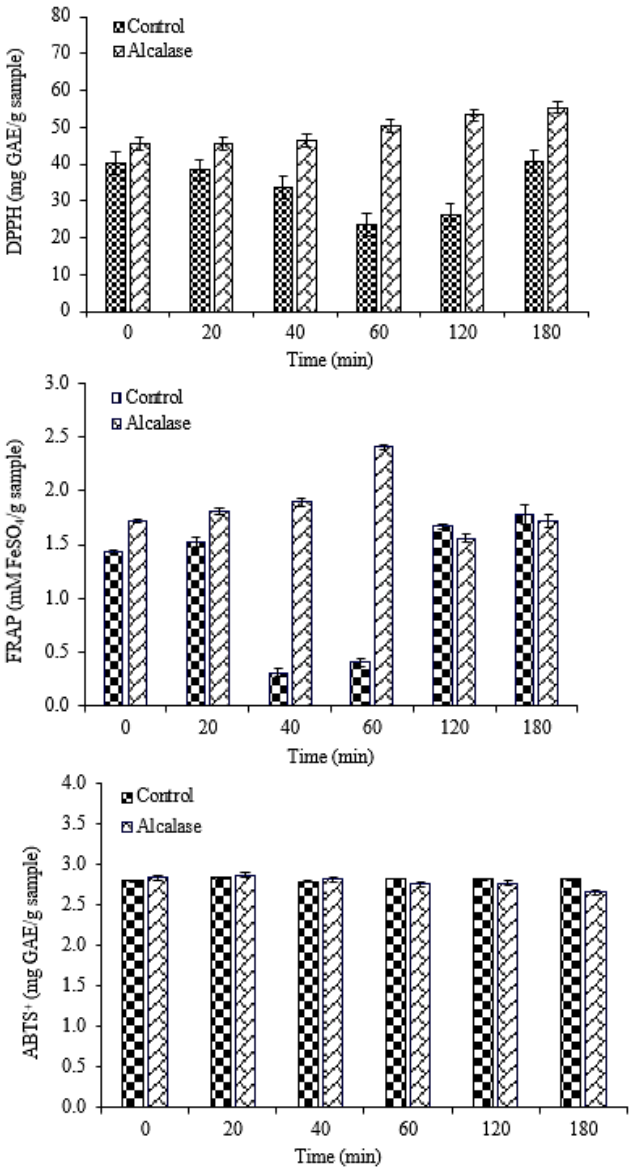

Fig. 2: Antioxidant activities of *G. bimaculatus* protein hydrolysates with/without alcalase at different times. The results of DPPH analysis (A), FRAP analysis (B), and ABTS^{+} analysis (C)

Effect of Hydrolysis Time on Antioxidant Activity

Figure 2 shows the antioxidant activities of CPH treated with and without alcalase hydrolysis at various times. Hydrolysis time significantly ($p<0.05$) impacted CPH DPPH and FRAP values in CPH but no significant differences in $ABTS^{\cdot+}$ values were found. The DPPH radical scavenging activity increased as alcalase hydrolysis time increased. CPH treated with alcalase gave the highest DPPH values at 60, 90, and 180 min (50.23, 53.25, and 55.43 mg GAE/g, respectively) while the control recorded the lowest value at 60 min (23.49 mg GAE/g). The FRAP values gradually increased with increasing alcalase hydrolysis times from 0 to 60 min, and then decreased after 120 and 180 min. The CPH treated with alcalase at 60 min had the highest FRAP value (2.40 mM $FeSO_4$/g), whereas CPH without the enzyme at 30 min had a low FRAP value (0.31 mM $FeSO_4$/g).

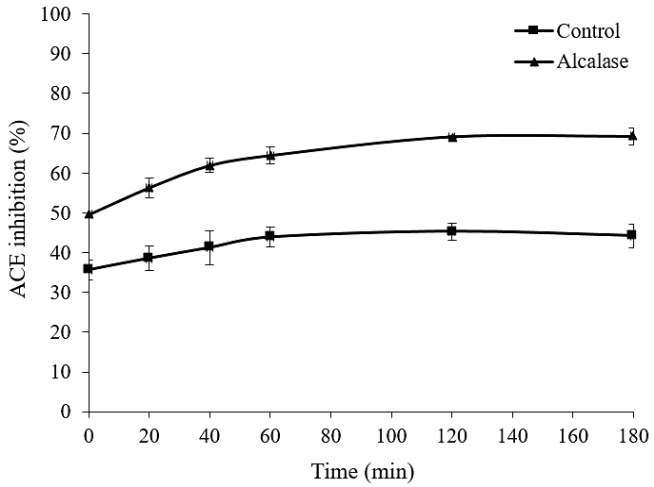

Fig.3: ACE inhibition percentages of *G. bimaculatus* protein hydrolyzed with/without alcalase at different times

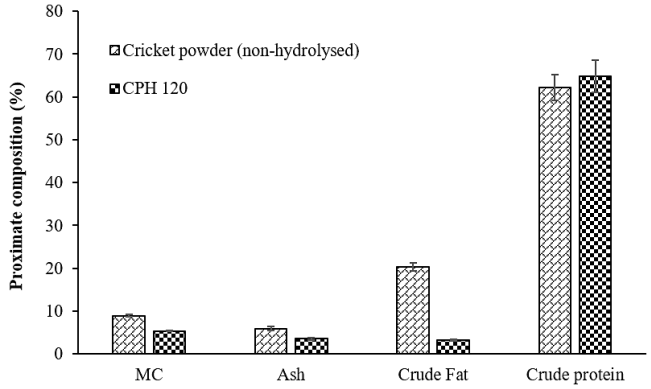

Fig.4: Proximate composition of cricket protein hydrolyzed at 120 min and unhydrolyzed cricket protein powder.

Effect of Hydrolysis Time on ACE Inhibition

The inhibition activity of ACE is one of the most common treatments for hypertension. Bioactive peptides derived from protein-rich foods are studied for their ability to reduce blood pressure by inhibiting ACE. Figure 3 shows that the ACE inhibition of CPH treated with and without alcalase at different times increased with increasing alcalase hydrolysis time. CPH treated with alcalase for 120 and 180 min showed the highest ACE inhibition values at 69.12 and 69.23%.

Proximate Composition of CPH

The CPH obtained from 120 min digestion time was chosen for proximate composition analysis. The crude protein and ash contents were higher than the control, while moisture and fat contents were lower. The crude protein content of CPH was 64.89%, and higher than the control (62.15%) (Figure 4).

Discussion

The DH is an indicator of peptide bond cleavage, with structured proteins degrading into smaller peptides.[4] Hydrolysis time significantly impacted the yield and DH. An increase in hydrolysis time enhanced the DH values of the cricket samples, resulting in a higher yield of CPH. The yield and DH of the control were lower than CPH because of no enzyme activity. Blanching as a pre-treatment and tray drying was used to inactivate the existing enzymes in *G. bimaculatus* cricket powder and denature its protein. Heating and water hydrolyzed the cricket powder, with increased yield and DH at 180 min of digestion. A heated cricket solution without enzyme addition has low DH (5.2%) because of protein denaturation and enzyme inactivation.[17] Cricket samples hydrolyzed with low enzyme concentration (0.5%) had lower DH, while samples treated with 3% enzyme concentration revealed high DH after 60 minutes.[9] Higher enzyme/substrate concentrations and longer hydrolysis times resulted in higher DH, while the control sample showed a low DH of 4.5%.[18] Similarly, hydrolysis of Gryllodes sigillatus proteins with 3% alcalase produced the highest DH value of 52%.[10] This study recorded the highest cricket proteins with alcalase hydrolysis after 120 minutes, indicating substrate active site enzyme saturation which prohibited further hydrolysis. However, DH values are impacted by several variables such as pH, temperature, hydrolysis time, and enzyme type and concentration. The functional and biological features of CPH products vary depending on the size, shape, amount of released peptides, and amino acid makeup.[19] Each increase in hydrolysis time gave higher protein and FFA contents compared to the control. This finding concurred with earlier research which reported that amino acid content varied.[10] In comparison to the control, some amino acid concentrations rose while others fell (for instance, Tyr and Arg levels were higher than the control). The FAA content (Val, Ile, Phe, Trp, Leu, and Tyr) that influenced taste was also improved by hydrolysis, especially tyrosine, which adds bitter or unpleasant flavors.[20] According to the research, utilizing protein hydrolysates in foods may cause issues with bitter peptide formation.[21] Foam development is controlled by molecular mobility, diffusion, and reorganization at the air-water interface. To be effective, foaming proteins must be able to quickly travel to the air-water contact, unfold, and rearrange at that interface, resulting in higher concentrations of amino acids during digestion. Amphiphilic proteins are also necessary for good surface characteristics. Different foam generation values are produced by the proteins and amino acid types found in each species of cricket. Hydrophilic amino acids are significant components of *G. bimaculatus*.[7] At the air-water contact, globular proteins showed reduced ability to unfold which diminished form foaming capacity.

Hydrolysis had no noticeable effect on the emulsion's capacity. CPH was found to be associated with significant enzymatic hydrolyzed protein and a reduction in emulsion activity.[22] Although greater interfacial activity was thought to be responsible for CPHs improved foaming and emulsion capabilities, hydrolysis also decreased the molecular weight of the polypeptides, and polypeptide size affected the interfacial layers' strength and network formation. Over time, hydrolysis weakens emulsions and foams' ability to stay stable. Although hydrolyzed cricket proteins showed good foaming properties in this experiment, there was no discernible effect on emulsion ability. Bread and other bakery goods have shown promise in using CPH as a foaming agent alternative. No apparent change in ABTS•+ was observed in CPH's antioxidant activity. DPPH and other peroxyl radicals are actively interacting with hydrophobic peptides, although ABTS•+ is more sensitive to hydrophilic peptides.[19] The antioxidant activity of peptides with low molecular weight increases because they expose more amino acids to interactions with free radicals, while enzymatic hydrolysis reactions break down large molecular weight peptides into smaller hydrophobic di- or tri- peptides with higher antioxidant capacity, resulting in higher antioxidant activity.[23] In CHP produced by enzymatic hydrolysis, some peptides contained amino acids (e.g., phenylalanine, histidine) associated with electron transfer capacity, resulting in increased antioxidant FRAP activity values. Protein hydrolysates have high reduced potential due to the availability of hydrogen protons and electrons generated by peptide bond cleavage.[24]

As reported in our previous study, *G. bimaculatus* cricket powder contains eight essential amino acids, six non-essential amino acids, and hydrophobic amino acids.[7] Protein hydrolysates high in hydrophobic amino acids are linked to the ability to scavenge or inhibit free radicals.[25]

His, Phe, Tyr, Trp, or Met undergo physical or chemical one-electron oxidation that inhibits amino acid formation.[26] Antioxidant substances including thiol groups reduce the sensitively of the FRAP technique.[27] Protein hydrolysates show antioxidant activity through lipid peroxidation, electron transfer, oxidative interactions with oxygen-containing chemicals, inhibition of free radicals, and metal ionization.[28] *G. bimaculatus* crickets are composed of amino acids and their protein hydrolysates show potential as bioactive substances with antioxidant activities.

Peptides derived from CPH hydrolyzed with alcalase exhibit strong in vitro ACE inhibition, particularly those with a higher DH. As a result, alcalase-treated CPH samples demonstrated superior ACE inhibition compared to the control sample. The ACEinhibition in CPH increased with DH reported by theprevious study.[18] The C-terminal amino acids with hydrophobic aromatic amino acid residues (Tyr, Phe, and Trp) or hydrophobic branched side chains (Val, Leu, and Ile) have an impact on the ACE inhibitory action.[29] These amino acids are usually associated with ACE-inhibitory activity and were found at the highest concentrations when hydrolyzed with 1.5% enzyme for 90 min.[9,30] The CPH obtained contained higher protein content than non-hydrolyzed crickets. The alcalase-hydrolyzed samples had higher protein content than the control.[9]

Conclusion

The alcalase treatment increased the biological activity of CPH from *G. bimaculatus*. Higher alcalase hydrolysis duration resulted in considerably higher CPH yields and DH. CPH treated with the enzyme for 120 and 180 min yielded the highest CPH yields (37.98 and 39.17 g/100 g, respectively) and the highest DH levels (84.78% and 84.42%). These durations also resulted in the greatest antioxidant activity (DPPH) and ACE inhibition activity when compared to the control (untreated CPH). The findings demonstrate that alcalase treatment is an effective technique for producing cricket-derived protein hydrolysates with enhanced biological activity. CPH showed promise for utilization in food formulations, with high foaming ability and foam stability. The development is driven by the need for sustainable food production methods to meet global protein demands while addressing sustainability concerns.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any materials that require ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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